Aliphatic side chain of catecholamine potentiates the stimulatory effect of the catechol part on the synthesis of nerve growth factor

Yoshiko Furukawa, Shoei Furukawa, Fumiaki Ikeda*, Eijiro Satoyoshi and Kyozo Hayashi+

*Division of Immunology, National Center of Nervous, Mental and Muscular Disorders, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187, *Institute of Biological Science, Mitsui Pharmaceuticals Inc., 1900-1 Togo, Mobara, Chiba 297* and +*Department of Pharmaceutics, Gifu Pharmaceutical University, Mitahora-Higashi, Gifu 502, Japan*

Received 11 September 1986

Catecholamines are potent in stimulating nerve growth factor (NGF) synthesis in mouse L-M cells. The relationship between the structure of catecholamines and their stimulatory effect on NGF synthesis has been studied using various 3,4-dihydroxyphenyl derivatives or their analogues. All 3,4-dihydroxyphenyl derivatives with two saturated carbons on the side chain were potent stimulators, whereas those with only one carbon on the side chain were weak stimulators. Drugs lacking the catechol ring were not effective. These results suggest that the catechol part of catecholamines is essential for the stimulatory effect and that the aliphatic side chain potentiates this effect. The present results also suggest the terminal amino residue on the side chain is not critical for the effect.

Nerve growth factor *Catecholamine* *(Mouse L-M cell)* *Adrenergic receptor* *Stimulatory effect* *Inducer*

1. INTRODUCTION

Nerve growth factor (NGF) is an essential protein for supporting growth and maintenance of peripheral sympathetic neurons as well as for facilitating the development of some sensory neurons during a brief period of early development [1]. Data have been recently accumulated that show NGF to be synthesized in sympathetically innervated end organs [2,3]. We have demonstrated that fibroblast cells cultured from various mouse organs [4,5] and established fibroblastic cell lines synthesize and secrete NGF [5,6]. These findings suggest that fibroblast cells are responsible for NGF synthesis in vivo. We have also demonstrated that catecholamines stimulate NGF synthesis in an established fibroblast cell line, the mouse L-M cell line [6]. 3-Dehydroxy precursors and 3-methoxy metabolites of catecholamines do not stimulate NGF synthesis, although pyrocatechol has a slight effect [6]. These results suggest that the stimulatory effect of catecholamines on NGF synthesis is based on the catechol part of the molecule and that the aliphatic side chain potentiates the effect. In the present study, we examined in further detail the relationship between the structure of catecholamines and their ability to stimulate NGF synthesis.

Abbreviations: NGF, nerve growth factor; EIA, enzyme immunoassay; BSA, bovine serum albumin; CM, conditioned medium; DOPA, 3,4-dihydroxyphenylalanine; DOPS, 3,4-dihydroxyphenylserine; DOPEG, 3,4-dihydroxyphenylglycol; DOPAC, 3,4-dihydroxyphenylacetic acid; DOMA, 3,4-dihydroxymandelic acid; DHBA, 3,4-dihydroxybenzylamine

2. MATERIALS AND METHODS

2.1. *Materials*

Mouse submaxillary gland β-NGF and anti-β-NGF antiserum were prepared as reported [7].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

Medium 199 was obtained from Flow Laboratories; peptone from Difco; tissue culture vessels from Falcon; BSA from Armour; 2,3-dihydroxypyridine, 3,4-dihydroxybenzaldehyde, DHBA, DOPS, 3,4-dihydroxynaphthalene, 3,4-dihydroxybenzoic acid, DOMA, 3,4-dihydroxycinnamic acid, DOPEG and DOPAC from Tokyo Kasei; pyrocatechol, (−)-epinephrine, epinine (deoxyepinephrine), dopamine, L-DOPA and (−)-isoproterenol from Sigma; (−)-norepinephrine from Nakarai Chemicals.

2.2. *Cell culture*

Mouse L-M cells were obtained from the American Type Culture Collection and maintained as monolayer cultures in Medium 199 supplemented with 0.5% peptone as described before [6]. To study the effect of catecholamines or other drugs, we inoculated cells into 24-well plates (well surface, 2.1 cm^2) at a cell density of 2–4 × 10^4 cells/well and cultured them for 3 days in peptone-containing medium. Then the medium was changed to Medium 199 containing 0.5% BSA with or without any drugs, and the cells were cultured for 24 h.

2.3. *Two-site EIA*

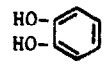
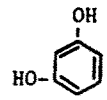
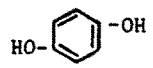
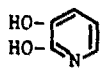
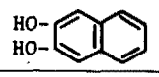
Two-site EIA for NGF was done as described [6,7]. Catecholamines and other drugs used in this study, as well as the unconditioned medium, did not affect the EIA system. Therefore, the CM was used directly in the EIA.

3. RESULTS

Catecholamines consist of catechol ring and aliphatic side chain. As we had already found that pyrocatechol (catechol or *o*-dihydroxybenzene) is slightly effective in stimulating NGF synthesis [6], we examined the effect of its analogues (table 1). Cells were cultured for 24 h in the presence of the drugs at a concentration of 0.1 or 0.2 mM, and then the NGF content of the medium of each culture was determined. Since NGF synthesized by L-M cells is secreted rapidly into the medium, the measurement of NGF content in the medium was

Table 1

Effects of pyrocatechol and its analogues on the NGF content in the medium of mouse L-M cells

Treatment		Structure	-Fold increase in NGF content
None (control)			1.00 ± 0.07
Pyrocatechol	0.1 mM		2.01 ± 0.09
	0.2 mM		2.31 ± 0.38
Resorcinol	0.1 mM		0.96 ± 0.02
	0.2 mM		1.16 ± 0.07
Hydroquinone	0.1 mM		1.47 ± 0.35
	0.2 mM		0.46 ± 0.22
2,3-Dihydroxypyridine	0.1 mM		1.16 ± 0.14
	0.2 mM		1.44 ± 0.05
2,3-Dihydroxynaphthalene	0.1 mM		0.85 ± 0.12
	0.2 mM		0.69 ± 0.08

Cells were cultured for 24 h with the indicated drugs. The NGF content is expressed as -fold increase over that in the absence of a given drug. Data are presented as means ± SE of four determinations

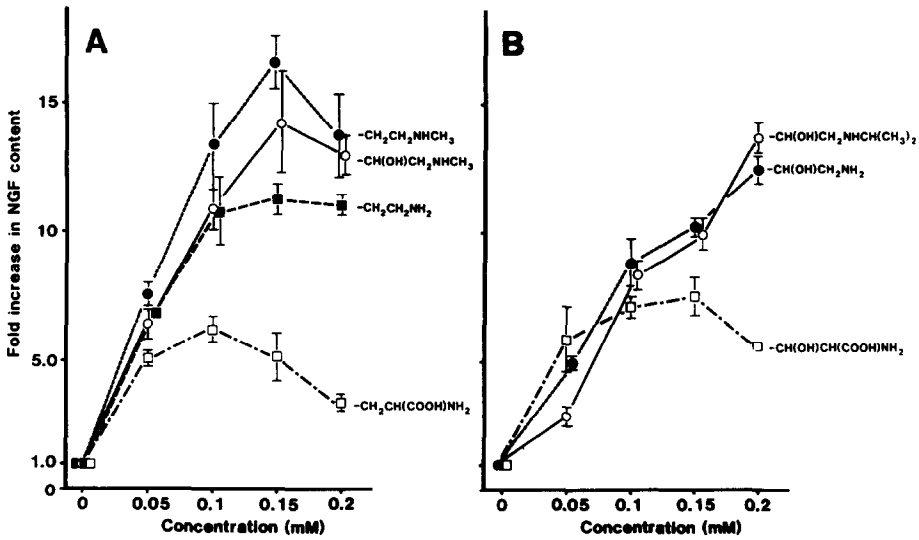


Fig.1. Effects of catecholamines and their analogues on the NGF content in the medium of mouse L-M cells. Cells were incubated for 24 h with various concentrations of (A) epinine (●), epinephrine (○), dopamine (■), DOPA (□); (B) isoproterenol (○), norepinephrine (●), DOPS (□). The structures of their side chain are indicated in the figure. The NGF content in the medium was determined by EIA and expressed as -fold increase over that in the absence of a given drug. Each point is the mean ± SE of four determinations.

considered to reflect the amount of NGF synthesized [6]. Both resorcinol, which has the phenolic dihydroxy group in the *meta*-position, and hydroquinone, with the group in the *para*-position, were ineffective. Also, neither 2,3-dihydroxynaphthalene, which is dihydroxylated on the naphthalene ring, nor 2,3-dihydroxypyridine, dihydroxylated on the pyridine ring instead of the benzene ring, was effective. These results indicated that the catechol ring is essential for the stimulatory effect on NGF synthesis.

In order to examine the relationship between the structure of the aliphatic side chain of catecholamines and their stimulatory effect on NGF synthesis, we cultured cells for 24 h in the presence of various concentrations of epinephrine, norepinephrine, dopamine, epinine, isoproterenol, or their analogous amino acids (DOPA and DOPS). As shown in fig.1, all drugs tested here induced an increase in the NGF content of the CM in a dose-dependent manner, but to various degrees. The structure-effect relationship among them may be summarized as follows: (i) β-Hydroxylation decreased the stimulatory effect on NGF synthesis. That is, epinine was more effective than epinephrine, and the effective concentration of dopamine was lower than that of norepinephrine. (ii) *N*-substitution enhanced the effect, that is, epinine and epinephrine were more effective than dopamine and norepinephrine, respectively. But the drug with a bulky substituent, such as the isopropyl group, reduced the effect. Namely, isoproterenol was less effective than epinephrine. (iii) α-Carboxylation resulted in a decrease in the effect. DOPA and DOPS were less effective than dopamine and norepinephrine, respectively.

Other 3,4-dihydroxyphenyl derivatives were also tested for their effects on NGF synthesis. As shown in fig.2, DOPEG, which was substituted with a hydroxy residue instead of the amino residue of norepinephrine, was effective, and its effect was almost the same as that of norepinephrine (fig.1). The effect of 3,4-dihydroxyphenylpropionic acid which has a carboxy residue instead of the amino residue of dopamine, was more effective than dopamine (figs 1 and 2). These results indicate that the amino residue of the catecholamine is not essential for the stimulatory effect.

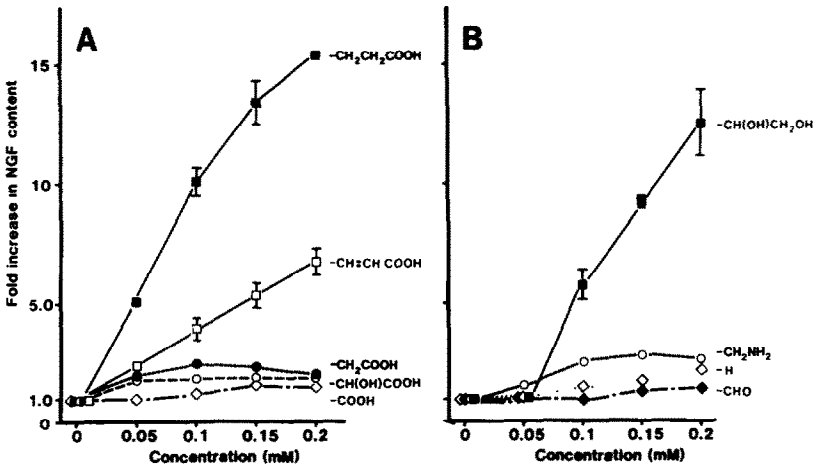


Fig.2. Effects of drugs with catechol part on the NGF content in the medium of mouse L-M cells. The experimental conditions were the same as those described in fig.1. (A) 3,4-Dihydroxyphenylpropionic acid (■), 3,4-dihydroxycinnamic acid (□), DOPAC (●), DOMA (○), 3,4-dihydroxybenzoic acid (◇); (B) DOPEG (■), DHBA (○), 3,4-dihydroxybenzaldehyde (◆), pyrocatechol (◇).

Fig.2 shows that shortening of the chain length resulted in a progressive reduction of the stimulatory effect on NGF synthesis. The effect of the drugs with 1 carbon on the side chain (DHBA, DOPAC, and DOMA) was markedly weaker than that of the drugs with 2 carbons (dopamine and 3,4-dihydroxyphenylpropionic acid). The drugs without the side chain (3,4-dihydroxybenzoic acid, and 3,4-dihydroxybenzaldehyde) were ineffective. 3,4-Dihydroxycinnamic acid, which has an unsatured side chain, was also effective, but it was not so potent as 3,4-dihydroxyphenylpropionic acid. These results indicate that 3,4-dihydroxyphenyl derivatives with side chains containing two saturated carbons are the most potent inducers of NGF synthesis.

4. DISCUSSION

The present study was aimed at confirming the suggestion that the stimulatory effect of catecholamines on NGF synthesis is based on the catechol part and is potentiated by the side chain. In order to confirm the necessity of the catechol part, we tested the effect on NGF synthesis of pyrocatechol, resorcinol, hydroquinone, 2,3-dihydroxypyridine, and 2,3-dihydroxynaphthalene. In order to examine the function of the side chain, we tested the effect on NGF synthesis of various 3,4-dihydroxyphenyl derivatives. The results presented here have confirmed the necessity of the catechol part (table 1) and of a side chain with two saturated carbons (figs 1 and 2). The present results also indicate that the terminal amino residue of catecholamines is not essential for their stimulation of NGF synthesis.

The mechanism by which catecholamines stimulate NGF synthesis is unknown. In a previous paper, we showed that this stimulatory effect is not likely to be mediated by α- or β-adrenergic receptors [6]. We suggested that norepinephrine and epinephrine are taken up into cells [8] and then trigger some yet unidentified intracellular reaction(s) [6]. It will be of great interest in the future to determine whether there is a correlation between the amount of a 3,4-dihydroxyphenyl derivative transported or permeated into cells and the degree of stimulation of NGF synthesis.

We previously suggested [6] that norepinephrine may well stimulate NGF synthesis in end organs in vivo as well as in vitro, because the concentration of norepinephrine in the vicinity of sympathetic nerve terminals was estimated to be sufficiently high [9]. Norepinephrine is synthesized in sympathetic nerves from tyrosine through DOPA and dopamine [10] and then released from nerve terminals. The possibility that DOPA and/or dopamine released from sympathetic nerve ter-

minals may be used for the stimulation of NGF synthesis is considered to be extremely low. After norepinephrine acts as a neurotransmitter on receptors of end organs, most of it is taken up again into the neurons, while another portion is metabolized in end organs and passed into the blood. Normetanephrine, DOMA and DOPEG are intermediates and 3-methoxy-4-hydroxymandelic acid is the major urinary excretion product [11]. The 3,4-dihydroxyphenyl metabolites such as DOPEG and/or DOMA might play an important role in stimulating NGF synthesis in the end organs. In any event, the scheme is very attractive that, for their normal growth and maintenance of function, peripheral sympathetic neurons depend on the NGF synthesized in sympathetically innervated end organs and that, conversely, this synthesis is regulated by neuronal activity through the neurotransmitter. These results also present the possibility that catecholamines or their analogues may be used in the future as inducers of NGF synthesis in vivo.

ACKNOWLEDGEMENT

This work was supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

[1] Levi-Montalcini, R. and Angeletti, P.U. (1968) Physiol. Rev. 48, 534–569.
[2] Shelton, D.L. and Reichardt, L.F. (1984) Proc. Natl. Acad. Sci. USA 81, 7951–7955.
[3] Heumann, R., Korsching, S., Scott, J. and Thoenen, H. (1984) EMBO J. 3, 3183–3189.
[4] Furukawa, Y., Furukawa, S., Satoyoshi, E. and Hayashi, K. (1984) J. Biol. Chem. 259, 1259–1264.
[5] Hayashi, K., Furukawa, Y., Furukawa, S. and Satoyoshi, E. (1985) Natural Products and Biological Activities (Imura, H. et al. eds) pp.251–261.
[6] Furukawa, Y., Furukawa, S., Satoyoshi, E. and Hayashi, K. (1986) J. Biol. Chem. 261, 6039–6047.
[7] Furukawa, S., Kamo, I., Furukawa, Y., Akazawa, S., Satoyoshi, E., Itoh, K. and Hayashi, K. (1983) J. Neurochem. 40, 734–744.
[8] Iversen, L.I. (1965) Br. J. Pharmacol. Chemother. 25, 18–33.
[9] Iversen, L.I. (1967) The Uptake and Storage of Noradrenaline in Sympathetic Nerves, pp.38–40.
[10] Goodall, McC. and Kirshner, N. (1958) Circulation 17, 366–371.
[11] Axelrod, J., Inscoe, J.K., Senoh, S. and Witkop, B. (1958) Biochim. Biophys. Acta 27, 210–211.